

CLAIMS

1. An isolated nucleic acid encoding a CTLA4-immunoglobulin fusion protein.
5 the nucleic acid comprising a nucleotide sequence encoding a first peptide having a CTLA4 activity and a nucleotide sequence encoding a second peptide comprising an immunoglobulin constant region which is modified to reduce at least one constant region-mediated biological effector function.
- 10 2. An isolated nucleic acid of claim 1, wherein the first peptide comprises an extracellular domain of the CTLA4 protein.
3. An isolated nucleic acid of claim 2, wherein the first peptide comprises amino acid residues 1-125 of the human CTLA4 protein.
- 15 4. An isolated nucleic acid of claim 2, wherein the first peptide binds B7-1 or B7-2.
5. An isolated nucleic acid of claim 1, wherein the immunoglobulin constant region comprises a hinge region, a CH2 domain and a CH3 domain.
- 20 6. An isolated nucleic acid of claim 5, wherein the hinge region, the CH2 domain and the CH3 domain are selected from the group consisting of C γ 1, C γ 2, C γ 3 and C γ 4.
- 25 7. An isolated nucleic acid encoding a CTLA4-immunoglobulin fusion protein, the nucleic acid comprising a nucleotide sequence encoding a first peptide having a CTLA4 activity and a nucleotide sequence encoding a second peptide comprising an immunoglobulin constant region wherein the immunoglobulin constant region comprises a heavy chain CH1 domain, a hinge region, a CH2 domain and a CH3 domain.
- 30 8. The isolated nucleic acid of claim 7, wherein the immunoglobulin constant region is modified to reduce at least one constant region-mediated biological effector function.
- 35 9. An isolated nucleic acid of claim 7, wherein the first peptide having a CTLA4 activity and the hinge region of the second peptide include at least one cysteine residue available for disulfide bond formation.

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10. The isolated nucleic acid of claim 8, wherein the first peptide having a CTLA4 activity and the hinge region of the second peptide include at least one cysteine residue available for disulfide bond formation.

11. An isolated nucleic acid of claim 5, wherein the biological effector function is selected from the group consisting of complement activation, Fc receptor interaction, and complement activation and Fc receptor interaction.

12. An isolated nucleic acid of claim 11, wherein at least one amino acid residue selected from a hinge link region of the CH2 domain is modified by substitution, addition or deletion.

13. An isolated nucleic acid of claim 12, wherein the at least one amino acid residue of the hinge link region of the CH2 domain is located at a position of a full-length intact immunoglobulin heavy chain selected from the group consisting of position 234, position 235 and position 237.

14. An isolated nucleic acid of claim 13, wherein the CH2 domain is derived from C γ 1.

15. An isolated nucleic acid of claim 14, wherein the at least one amino acid residue selected from a hinge link region of the CH2 domain is modified by at least one substitution selected from the group consisting of: substitution of Leu at position 234 with Ala; substitution of Leu at position 235 with Glu; and substitution of Gly at position 237 with Ala.

16. An isolated nucleic acid of claim 15, wherein Leu at position 234 is substituted with Ala, Leu at position 235 is substituted with Glu and Gly at position 237 is substituted with Ala.

17. An isolated nucleic acid of claim 13, wherein the CH2 domain is derived from C γ 4.

18. An isolated nucleic acid of claim 17, wherein the at least one amino acid residue selected from a hinge link region of the CH2 domain is modified by at least one substitution selected from the group consisting of: substitution of Leu at position 234 with Ala; substitution of Leu at position 235 with Glu; and substitution of Gly at position 237 with Ala.

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19. An isolated nucleic acid of claim 18, wherein Leu at position 235 is substituted with Glu and Gly at position 237 is substituted with Ala.

5 20. An isolated nucleic acid of claim 11, wherein at least one amino acid residue selected from a hinge-proximal bend region of the CH2 domain is modified by substitution, addition or deletion.

10 21. An isolated nucleic acid of claim 20, wherein an amino acid residue at position 331 of an intact immunoglobulin heavy chain is modified by substitution with another amino acid residue.

15 22. An isolated nucleic acid of claim 21, wherein the CH2 domain is derived from C γ 1 C γ 2, C γ 3, or C γ 4.

23. An isolated nucleic acid of claim 21, wherein Pro at position 331 of an intact immunoglobulin heavy chain is substituted with Ser.

20 24. An isolated nucleic acid of claim 11, wherein at least one amino acid residue of the CH2 domain located at a position of an intact immunoglobulin heavy chain selected from the group consisting of position 318, position 320 and position 322 is modified by substitution, addition or deletion.

25 25. An isolated nucleic acid of claim 24, wherein the at least one amino acid residue of the CH2 domain is modified by at least one substitution selected from the group consisting of: substitution of Glu at position 318 with Ala or Val; substitution of Lys at position 320 with Ala or Gln; and substitution of Lys at position 322 with Ala or Gln.

30 26. An isolated nucleic acid of claim 25, wherein Glu at position 318 is substituted with Ala or Val, Lys at position 320 is substituted with Ala or Gln and Lys at position 322 is substituted with Ala or Gln.

35 27. An isolated nucleic acid of claim 5, wherein the hinge region is modified to reduce at least one biological effector function.

28. An isolated nucleic acid of claim 27, wherein the biological effector function is complement activation.

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29. An isolated nucleic acid of claim 28, wherein at least one amino acid residue located in the hinge region is modified by substitution, addition or deletion.

30. An isolated nucleic acid of claim 29, wherein the immunoglobulin constant region is C γ 1 C γ 2, C γ 3, or C γ 4.

31. An isolated nucleic acid of claim 30, wherein the hinge region of C γ 1 or C γ 3 is substituted with a hinge region derived from C γ 4.

32. An isolated nucleic acid of claim 5, wherein the CTLA4-immunoglobulin fusion protein comprises an amino acid sequence shown in SEQ ID NO: 26.

33. An isolated nucleic acid of claim 5 comprising a nucleotide sequence shown in SEQ ID NO: 25.

34. An isolated nucleic acid of claim 5, wherein the CH2 domain is modified by substitution of Glu for Leu at position 235 of an intact immunoglobulin heavy chain and by substitution of Ala for Gly at position 237 of an intact immunoglobulin heavy chain.

35. An isolated nucleic acid of claim 34, wherein the CTLA4-immunoglobulin fusion protein comprises an amino acid sequence shown in SEQ ID NO: 28.

36. An isolated nucleic acid of claim 34 comprising a nucleotide sequence shown in SEQ ID NO: 27.

37. An isolated nucleic acid of claim 5, wherein the CTLA4-immunoglobulin fusion protein comprises an amino acid sequence shown in SEQ ID NO: 24.

38. An isolated nucleic acid of claim 5 comprising a nucleotide sequence shown in SEQ ID NO: 23.

39. An isolated nucleic acid encoding a CTLA4-immunoglobulin light chain fusion protein, wherein the nucleic acid comprises a nucleotide sequence encoding a first peptide comprising a CTLA4 extracellular domain and a nucleotide sequence encoding a second peptide comprising an immunoglobulin light chain constant domain.

40. An isolated nucleic acid capable of expression in a bacterial host cell, the nucleic acid consisting of a nucleotide sequence encoding a CTLA4 extracellular domain.

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41. An isolated nucleic acid comprising a nucleotide sequence encoding a soluble CTLA4 protein capable of expression in a bacterial host cell, wherein the nucleic acid consists of a nucleotide sequence encoding a signal sequence and a nucleotide sequence
5 encoding a CTLA4 extracellular domain.

42. A recombinant expression vector comprising a nucleic acid of claim 1.

43. A recombinant expression vector comprising a nucleic acid of claim 3.

44. A recombinant expression vector comprising a nucleic acid of claim 7.

45. A recombinant expression vector comprising a nucleic acid of claim 8.

46. A recombinant expression vector comprising a nucleic acid of claim 39.

47. A recombinant expression vector comprising a nucleic acid of claim 40.

48. A recombinant expression vector comprising a nucleic acid of claim 41.

49. A host cell transfected with the expression vector of claim 38 capable of directing the expression of a CTLA4-immunoglobulin fusion protein.

50. A host cell transfected with the expression vector of claim 43 capable of directing the expression of a CTLA4-immunoglobulin fusion protein.

51. A host cell transfected with the expression vector of claim 7 capable of directing the expression of a CTLA4-immunoglobulin fusion protein.

52. A host cell transfected with the expression vector of claim 8 capable of directing the expression of a CTLA4-immunoglobulin fusion protein.

53. A host cell transfected with the expression vector of claim 46 capable of directing the expression of a CTLA4-immunoglobulin fusion protein.

54. A bacterial host cell transfected with the expression vector of claim 47 capable of directing the expression of a CTLA4 extracellular domain.

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55. A bacterial host cell transfected with the expression vector of claim 48 capable of directing the expression of a CTLA4 extracellular domain.

56. A CTLA4-immunoglobulin fusion protein comprising a first peptide having a CTLA4 activity and a second peptide comprising an immunoglobulin constant region which is modified to reduce at least one constant region-mediated biological effector function relative to a CTLA4-IgG1 fusion protein.

57. A CTLA4-immunoglobulin fusion protein of claim 56, wherein the first peptide comprises an extracellular domain of the CTLA4 protein.

58. A CTLA4-immunoglobulin fusion protein of claim 57, wherein the first peptide comprises amino acid residues 1-125 of the human CTLA4 protein.

59. A CTLA4-immunoglobulin fusion protein of claim 56, wherein the immunoglobulin constant region comprises a hinge region, a CH2 domain and a CH3 domain.

60. A CTLA4-immunoglobulin fusion protein of claim 59, wherein the hinge region, the CH2 domain and the CH3 domain are selected from the group consisting of C γ 1, C γ 2, C γ 3 and C γ 4.

61. A CTLA4-immunoglobulin fusion protein, comprising a first peptide having a CTLA4 activity and a second peptide comprising an immunoglobulin constant region wherein the immunoglobulin constant region comprises a heavy chain CH μ domain, a hinge region, a CH2 domain and a CH3 domain.

62. The peptide of claim 61, wherein the immunoglobulin constant region is modified to reduce at least one constant region-mediated biological effector function.

63. The peptide of claim 61 wherein the first peptide having a CTLA4 activity and the hinge region of the second peptide include at least one cysteine residue available for disulfide bond formation.

64. The isolated nucleic acid of claim 62, wherein the first peptide having a CTLA4 activity and the hinge region of the second peptide include at least one cysteine residue available for disulfide bond formation.

65. A CTLA4-immunoglobulin fusion protein of claim 59, wherein the CH2 domain is modified to reduce biological effector functions.

66. A CTLA4-immunoglobulin fusion protein of claim 65, wherein the biological effector function is selected from the group consisting of complement activation, Fc receptor interaction, and complement activation and Fc receptor interaction.

67. A CTLA4-immunoglobulin fusion protein of claim 66, wherein the CH2 domain is modified by substitution of an amino acid residue located at a position of an intact immunoglobulin heavy chain selected from the group consisting of position 234, position 235 and position 237.

68. A CTLA4-immunoglobulin fusion protein of claim 67 comprising an amino acid sequence shown in SEQ ID NO: 24.

69. A CTLA4-immunoglobulin fusion protein of claim 68 comprising an amino acid sequence shown in SEQ ID NO: 28.

70. A CTLA4-immunoglobulin light chain fusion protein, wherein the first peptide comprises a CTLA4 extracellular domain and the second peptide comprises an immunoglobulin kappa light chain constant domain.

71. An isolated peptide consisting of a CTLA4 extracellular domain produced by a bacterial host cell of claim 54.

72. An isolated peptide consisting of a signal sequence and a CTLA4 extracellular domain produced by a bacterial host cell of claim 55.

73. A composition suitable for pharmaceutical administration comprising a CTLA4-immunoglobulin fusion protein of claim 56, and a pharmaceutically acceptable carrier.

74. A composition suitable for pharmaceutical administration comprising a CTLA4-immunoglobulin fusion protein of claim 58, and a pharmaceutically acceptable carrier.

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FIGURE 5
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75. A method for producing a CTLA4-immunoglobulin fusion protein, comprising culturing a host cell of claim 49 in a medium to express the protein and isolating the protein from the medium.

5 76. A method for producing a CTLA4-immunoglobulin fusion protein, comprising culturing a host cell of claim 50 in a medium to express the protein and isolating the protein from the medium.

10 77. A method for producing a CTLA4-immunoglobulin fusion protein, comprising culturing a host cell of claim 54 in a medium to express the protein and purifying the protein from inclusion bodies.

15 78. A method for producing a CTLA4-immunoglobulin fusion protein, comprising culturing a host cell of claim 55 in a medium to express the protein and purifying the protein by release from periplasm.

20 79. A method for inhibiting an interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell comprising contacting the antigen presenting cell with a CTLA4-immunoglobulin fusion protein of claim 56.

80. A method for inhibiting an interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell comprising contacting the antigen presenting cell with a CTLA4-immunoglobulin fusion protein of claim 58.

25 81. A method for treating an autoimmune disease in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 56.

30 82. A method for treating an autoimmune disease in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 62.

35 83. A method for treating an autoimmune disease in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 70.

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84. A method for treating an autoimmune disease in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 71.

85. A method for treating an autoimmune disease in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 72.

86. A method of claim 81, wherein the autoimmune disease is selected from the group consisting of diabetes mellitus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erahmatosis, and autoimmune thyroiditis.

87. A method for treating allergy in a subject mediated by interaction of a CTLA4 ligand on an antigen presenting cell with a receptor for the CTLA4 ligand on a T cell, comprising administering to the subject a CTLA4-immunoglobulin fusion protein of claim 56.

88. A method for inhibiting graft-versus-host disease (GVHD) in a bone marrow transplant recipient, comprising administering to the recipient a CTLA4-immunoglobulin fusion protein of claim 56.

89. A method of claim 88, wherein donor bone marrow is contacted with the CTLA4-immunoglobulin fusion protein and with cells from the transplant recipient *ex vivo* prior to transplantation of the donor bone marrow into the recipient.

90. A method for inhibiting rejection of transplanted cells in a transplant recipient, comprising administering to the recipient a CTLA4-immunoglobulin fusion protein of claim 56.

91. A method for identifying molecules which inhibit binding of CTLA4 to a CTLA4 ligand, comprising

a) contacting the CTLA4-immunoglobulin fusion protein of claim 56 with:

- i) a CTLA4 ligand, and
- ii) a molecule to be tested.

wherein either the CTLA4-immunoglobulin fusion protein or the CTLA4 ligand is labeled with a detectable substance:

- b) removing either unbound CTLA4-immunoglobulin fusion protein or unbound CTLA4 ligand; and
- 5 c) determining the amount of CTLA4-immunoglobulin fusion protein bound to the CTLA4 ligand.

wherein a reduction in the amount of CTLA4-immunoglobulin fusion protein bound to the CTLA4 ligand in the presence of the molecule indicates that the molecule inhibits binding of CTLA4 to the CTLA4 ligand.

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